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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

SERUM TRAIL VE TRAIL RESEPTÖRLERİNİN MEME KANSERİNDEKİ POTANSİYEL ROLÜNÜN ARAŞTIRILMASI

INVESTIGATION OF THE POTENTIAL ROLE OF SERUM TRAIL AND TRAIL RECEPTORS IN BREAST CANCER

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ÖZET

AMAÇ: Apoptoz, normal hücresel homeostazın sürdürülmesinde kritik bir rol oynar ancak meme kanserinde sıklıkla düzensizlik gösterebilir. Bu çalışma, meme kanseri hastaları ile kontrol grubunda TRAIL, DR4 ve DR5'in serum düzeylerini değerlendirmeyi amaçlamaktadır. Ayrıca, meme kanserinde enflamatuvar ve apoptotik belirteçlerin kapsamlı bir analizini sağlamak için CCL2 ve CCL5'in serum seviyeleri de ölçülecektir.

GEREÇ VE YÖNTEM: Bu vaka-kontrol çalışması, Gazi Üniversitesi Tıp Fakültesi Hastanesi genel cerrahi servisinde kayıtlı, yaşları uyumlu 62 meme kanseri hastası ve 62 kontrol grubundan oluşmaktadır. Serum parametreleri prospektif olarak Enzim Bağlantılı İmmünosorbent Test (ELISA) ile ölçülmüştür.

BULGULAR: Çalışma, meme kanseri hastalarında kontrol grubuna kıyasla serum DR4 ve DR5 düzeylerinde anlamlı artışlar olduğunu ortaya koymuştur (6,99±1,05'e karşı 4,61±0,61 ng/mL, p=0,003; 1,57±0,12'ye karşı 1,03±0.10 ng/mL, p<0,001, sırasıyla). Serum TRAIL, CCL2 ve CCL5 düzeyleri açısından iki grup arasında anlamlı bir fark gözlenmemiştir. Tümör boyutu 2 cm'den büyük olan hastalarda serum CCL2 düzeyi anlamlı derecede daha yüksekti (p=0,007). Ayrıca, hasta grubunda TRAIL ile DR5/CCL5 serum konsantrasyonları arasında pozitif bir korelasyon bulunmuştur.

SONUÇ: Meme kanseri hastalarında serum DR4 ve DR5 düzeylerinin yüksek bulunması, bu biyobelirteçlerin tanısal amaçla kullanılabileceğini düşündürmektedir. Ayrıca, TRAIL ile DR5 ve CCL5 arasındaki pozitif korelasyon, apoptoz ve bağışıklık yanıtı arasındaki olası bir etkileşimi göstermektedir. Bu ilişkilerin daha net anlaşılması için ileri çalışmalara ihtiyaç vardır.

ANAHTAR KELİMELER: TNF'ye bağlı apoptoz indükleyici ligand (TRAIL), Ölüm reseptörü 4 (DR4), Ölüm reseptörü 5 (DR5), Meme kanseri.

ABSTRACT

OBJECTIVE: Apoptosis is crucial for maintaining normal cellular homeostasis, but dysregulation may frequently be observed in breast cancer. This study aims to evaluate the serum levels of TRAIL, DR4, and DR5 in patients with breast cancer and the control group. Additionally, the serum levels of CCL2 and CCL5 will also be measured to provide a comprehensive analysis of inflammatory and apoptotic markers in breast cancer.

MATERIAL AND METHODS: This case-control study consists of 62 breast cancer patients and 62 age-matched controls, enrolled in general surgery service at the Gazi University Medical Faculty Hospital. Serum parameters were measured prospectively via Enzyme-Linked Immunosorbent Assay.

RESULTS: Serum levels of DR4 and DR5 were significantly higher in breast cancer patients compared to the controls (6.99±1.05 versus 4.61±0.61 ng/mL, p=0.003; 1.57±0.12 versus 1.03±0.10 ng/mL, p<0.001, respectively). No statistically significant differences were observed between the two groups in TRAIL, CCL2 or CCL5 levels. However, serum CCL2 levels were significantly elevated in patients with a tumour size greater than 2 cm (p=0.007). Moreover, a positive correlation was found between the serum concentrations of TRAIL and DR5/CCL5 in the patient group.

CONCLUSIONS: Elevated serum levels of DR4 and DR5 in breast cancer patients suggest their potential as diagnostic biomarkers. Additionally, the observed positive correlation between TRAIL and DR5, and CCL5 may reflect a complex interplay between apoptosis and immune response. Further studies are needed to better understand these relationships.

KEYWORDS: TNF-related apoptosis-inducing ligand (TRAIL), Death receptor 4 (DR4), Death receptor 5 (DR5), Breast cancer.

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INTRODUCTION

Breast cancer is a prevalent type of cancer that results in a substantial number of cancer-related fatalities among women globally. The high incidence and mortality rates underscore the critical need for improved diagnostic and therapeutic strategies.

Apoptosis is a fundamental process in the regulation of cellular homeostasis, ensuring the proper maintenance of cell populations and tissue development. Resistance to apoptosis is a hallmark of cancer, with alterations in several genes, including tumour suppressor genes, anti - apoptotic regulators, and pro - apoptotic regulators, contributing to this resistance. Dysregulation of apoptosis has been implicated in several pathological conditions, including cancer (1). Apoptosis dysregulation has also been linked to breast cancer development and progression (2).

The extrinsic apoptotic pathway is activated by several ligands, including FAS ligand, TNF-α, and TNF-related apoptosis-inducing ligand (TRAIL), binding to their respective death receptors (3). Death receptor 4 (DR4) and Death receptor 5 (DR5) are capable of inducing apoptosis via binding TRAIL. The binding initiates the formation of the death-inducing signalling complex (DISC), which leads to the activation of caspase-8 and downstream effector caspases. In normal conditions, this cascade results in programmed cell death. However, the expression levels of TRAIL receptors can be altered in cancer. Cancer cells can reduce the expression of DR4 and DR5, produce non-functional receptors that block the signal, or acquire impairments in the signalling pathway that follows receptor activation all of which help them avoid being eliminated by TRAIL-triggered cell death (2).

Despite the central role of TRAIL receptors in apoptosis, their behavior in cancer remains complex. While increased serum levels of DR4 and DR5 have been reported in patients with small-cell lung cancer (4) or severe ovarian cystadenoma (5), it remains uncertain whether the pathogenesis of cancer is associated with an increase or a decrease in serum TRAIL levels. While DR4 and DR5 have been studied in various cancers, their role in breast cancer has not been investigated. Chemokines can function similarly to growth factors by promoting metastasis formation, angiogenesis, and inducing an immunosuppressive microenvironment. The C-C motif chemokine ligand-5 (CCL5) and the C-C motif chemokine ligand-2 (CCL2) are key chemokines that actively modulate the tumour microenvironment by recruiting immune cells, particularly monocytes and macrophages. CCL2 and CCL5 attract tumour-associated macrophages (TAMs), which recruit circulating monocytes to the tissues and promote the production of immune-regulating and growth-promoting factors (6).

The interplay between TRAIL/DR4/DR5 and chemokines like CCL2 and CCL5 may contribute to the tumour microenvironment that balances immune suppression and evasion with apoptotic resistance. TRAIL has been shown to induce CCL2 secretion in an NF-KB-dependent manner *in vitro* and *in vivo*. TRAIL induces CXCL2 secretion in TRAIL-sensitive cells both *in vitro* and *in vivo* (7). Increased expression levels of CCL2 and CCL5 are observed in breast tumour cells compared to normal breast epithelial cells (8). This study aims to fill this gap by investigating the serum levels of TRAIL, DR4, and DR5 in breast cancer patients. Furthermore, our study aimed to investigate circulating levels of chemokines, CCL2 and CCL5 in breast cancer.

MATERIALS AND METHODS

Study Population

The current case-control study consists of 62 female patients recently diagnosed with breast cancer and 62 controls. The study included female patients over the age of 18 with a confirmed diagnosis of breast cancer. Age-matched healthy women without a history of cancer were enrolled as the control group. Participants were excluded based on medical history if they had any systemic disease such as diabetes mellitus, hypertension, or autoimmune disorders. Additional exclusion criteria included prior chemotherapy or radiotherapy, use of medications that could affect thyroid function, and pregnancy or lactation. All data regarding eligibility were obtained from the patients' medical records and clinical history. The control group consisted of 62 age-matched healthy women over the age of 18 with no known history of

cancer or chronic systemic diseases, based on available medical records. These individuals were selected from patients attending the hospital for routine, non-cancer-related visits. No additional laboratory or clinical assessments were conducted to rule out current infections or undiagnosed conditions. This study conducted prognostic evaluations on several parameters, including Ki67 proliferation, tumour size, hormone receptor status, as well as histological and molecular subtypes, within the patient group. The Modified Scarff-Bloom-Richardson classification was employed for histological grading of both invasive ductal and invasive lobular carcinoma. In addition, a survey was conducted on the study group to obtain information on factors such as age, gender, age at menarche, education level, and tobacco use.

Procedure

Peripheral venous blood samples were collected from all participants who were admitted to the Gazi University Faculty of Medicine Hospital General Surgery Outpatient Clinic between 2019 and 2020. A 5 mL blood sample was drawn into a red-top tube and all samples were centrifuged at 1000x g for 15 minutes at 4°C to separate the serum from the cells; then carefully aspirated using a pipette to a sterile eppendorf tube, which was labelled with the unique identifier. The serum samples were kept in -80°C freezer. Information regarding whether the blood samples were collected preoperatively or postoperatively was not available. The study employed the commercially available Enzyme-linked Immunosorbent Assay (ELISA) to measure the serum levels of TRAIL, DR4, DR5, CCL2, and CCL5 according to the manufacturer's instructions.

The following kits were used for this purpose: Shanghai Sunred (China) ELISA kit was used for DR4 and USCN Life Science Inc. (China) for TRAIL, DR5, CCL2, and CCL5. The repeatability and reproducibility of the method were assessed by replicating analyses of serum samples. The samples of serum were analysed twice a day to determine the intra-day repeatability expressed. Then, the analyses were repeated over two consecutive days to calculate inter-day reproducibility. Intra-assay coefficient of variability of TRAIL, DR4, DR5, CCL2, and CCL5, are respectively 5.4%, 1.2%, 5.3%, 4.1% and 1.7%. Inter-assay CV of TRAIL, DR4, DR5, CCL2 and CCL5 are respectively 7.8%, 9.0%, 9.0%, 9.3% and 8.8%.

Ethical Committee

The study will be carried out according to the standards outlined in the Declaration of Helsinki. Gazi University Clinical Research Ethics Committee approved (Date: 09.12.2019/ No: 255) this study and obtained informed consent from both patients and controls.

Statistical Analysis

The statistical analysis of the data was performed using the SPSS software package version 22.0 for Windows (IBM Corporation, Chicago, Illinois, USA). The normality of the distribution of variables was assessed using the Shapiro-Wilk normality test, while the equality of variances was assessed using the Levene test. To compare the two study groups, the Student's t-test and Mann-Whitney test were employed, whereas the ANOVA and Kruskal-Wallis test were used to compare more than two groups. Post-hoc analysis of pairwise comparisons between groups was conducted using the Tukey test. The significance of the correlations between variables was determined by calculating the Pearson correlation coefficient (r). The level of statistical significance for all analyses was set at P values < 0.05.

RESULT

The study groups were statistically compared based on age, BMI, age of menarche, and smoking status, and no significant difference was found between the patient and control groups **(Table 1)**.

Table 1: Demographic characteristics of the study groups

Characteristic		Breast Cancer Patients	Healthy Controls
N		62	62
Age (Mean ± SD)		53.82 ± 1.56	52.32 ± 1.37
Age Groups (N)	≤ 50 years	28	28
	> 50 years	34	34
BMI (Mean ± SD)		27.55 ± 0.6	28.4 ± 0.64
BMI Groups (N)	≤ 25	20	13
	> 25 and ≤ 30	25	32
	> 30	17	17
Age at Menarche (Mean ± SD)		13.21 ± 0.17	13.24 ± 0.19
Age at Menopause (Mean ± SD)		48.75 ± 0.81	47.92 ± 0.79
Menopause Status (N)	Premenopausal	25	25
	Postmenopausal	37	37
Family History of Cancer (N)	Yes	18	15
	No	44	47
Smoking Status (N)	Yes	11	20
	No	51	42

BMI= Body Mass Index

The majority of the patient group primarily had invasive ductal carcinoma (72.6%) with most being Luminal A subtype (58.1%). The histologic classification showed that most cases were grade 2 (43.5%), followed by grade 3 (37.1%). More than half of the patients (58.1%) had the Luminal A molecular subtype. Most cases were oestrogen receptor-positive (85.5%) and progesterone receptor-positive (77.4%). Ki-67 proliferation was ≤ 20% in 48.4% of patients, and tumour size was ≤2 cm in 46.8% of cases **(Table 2)**.

Table 2: Clinical characteristics of the patient group

Characteristic	N	%
Total	62	100
IDC/ILC /Mix Type/ Other	45/4/7/6	72.6/6.5/11.3/9.7
Luminal A/Luminal B/ HER2-enrich/ Basal-like	36/16/4/6	58.1/25.8/6.5/9.7
Grade I/II/ III	12/27/23	19.4/43.5/37.1/85.5
ER +/-	53/9	85.5/14.4
PR +/-	48/14	77.4/22.6
HER2 status +/-	20/42	32.3/67.7
Ki-67 proliferation ≤20% / >20%/ Unknown	30/27/5	48.4/43.6/8.1
Tumour size ≤2 cm/ >2 cm/ Unknown	29/25/8	46.8/40.3/12.9

IDC=Invasive Ductal Carcinoma, ILC=Invasive Lobular Carcinoma, HER2=Human Epidermal Growth Factor Receptor 2, ER=Oestrogen Receptor, PR=Progesterone Receptors

In this study, the serum levels of DR4 and DR5 were found to be significantly higher in the patient group as compared to the control group, as presented in **Table 3**. However, no statistically significant difference was observed between the two groups with respect to serum TRAIL, CCL2 and CCL5.

Table 3: Comparison of serum TRAIL, DR4, DR5, CCL2, and CCL5 levels among groups

Parameters	Patient group (X±SE) (N=62)	Control group (X±SE) (N=62)	P value
TRAIL (ng/mL)	9.07±0.56	10.15±0.50	0.079
DR4 (ng/mL)	**6.99±1.05***	4.61±0.61	**0.003**
DR5 (ng/mL)	**1.57±0.12***	1.03±0.10	**<0.001**
CCL2 (pg/mL)	82.8±4.40	100.24±5.33	0.05
CCL5 (ng/mL)	65.45±3.10	57.89±1.90	0.163

** Significant difference from the control group*

The analysis demonstrated a significant increase in the Ki67 proliferation proportion with increasing tumour grade (p<0.001). Additionally, Ki67 proliferation is lower in patients with hormone receptor-positive compared to those with ER- and PR- (p=0.014 and p=0.05 respectively). The relationship between clinical factors and breast cancer grade is demonstrated in **Figure 1**. The present study found a statistically significant difference in the age at menarche between patients with grade 3 breast cancer and those with grade 2 breast cancer. Specifically, patients with grade 3 breast cancer had a significantly lower age at menarche compared to their counterparts with grade 2 breast cancer (p=0.037).

The concentrations of serum TRAIL, DR4, DR5, CCL2, and CCL5 were assessed in conjunction with several clinical parameters, including tumour size, ER, PR, and HER status **(Table 4)**. The results revealed a statistically significant elevation in the level of serum CCL2 among individuals diagnosed with a tumour size exceeding 2 cm (p=0.007).

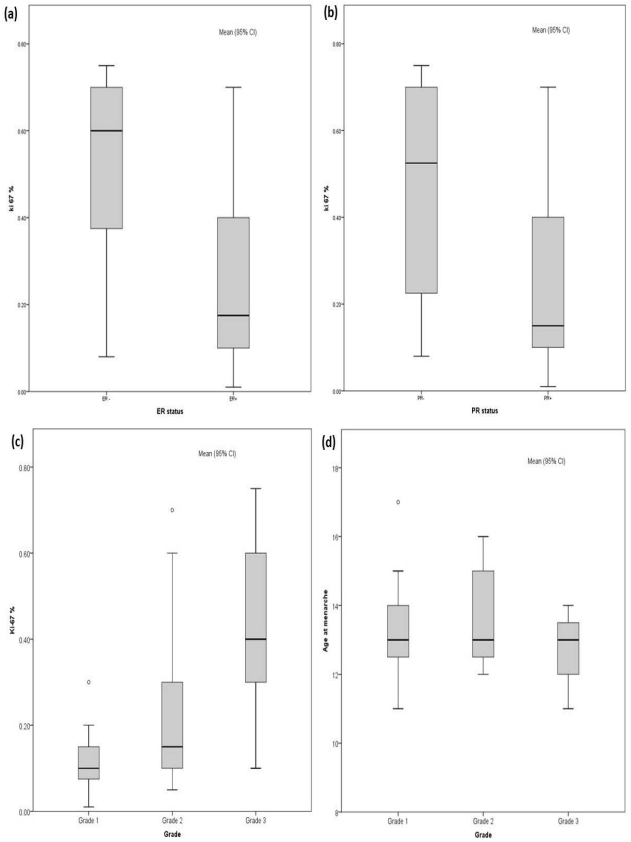

Figure 1: Evaluation of clinical factors in terms of breast cancer grade, and hormone receptor status. Ki67 proliferation was significantly higher in patients with ER- **(a)** or PR- **(b)** compared to those with ER+ or PR+. The figure illustrates a significant increase in the Ki67 proliferation with increasing tumour grade **(c)** and a significantly low mean of age at menarche in advanced-grade patients **(d)**.

Table 4: Evaluation of parameters in terms of some prognostic factors such as tumour size, ER, PR, and HER-2 status

	N	TRAIL (ng/mL)	DR4 (ng/mL)	DR5 (ng/mL)	CCL2 (pg/mL)	CCL5 (ng/mL)
Grade1	12	9.71±1.64	4.02±0.63	1.37±0.27	88.61±11.25	75.75±9.26
Grade2	27	8.75±0.83	7.37±1.41	1.74±0.15	76.37±6.06	69.27±4.88
Grade3	23	9.1±0.83	8.09±2.26	1.48±0.22	87.33±7.55	**55.59±2.98***
Tumour ≤2 cm	29	8.92±0.82	5.73±1.01	1.33±0.10	69.83±6.32	63.80±7.50
Tumour >2 cm	25	8.51±0.79	7.33±1.80	1.63±0.11	**95.21±32.21****	65.47±4.43
ER +	53	8.59±0.61	6.53±1.15	1.49±0.13	82.8±4.81	65.29±3.88
ER –	9	10.06±1.09	7.96±2.23	1.73±0.22	82.7±10.80	65.79±5.26
PR +	48	8.75 ±0.61	7.3±1.28	1.53±0.10	83.27±5.11	66.77±3.78
PR –	14	10.13 ±1.15	5.93±1.67	1.7±0.25	81.18±8.85	60.92±4.76
HER-2 +	20	10.06 ±1.03	7.96±2.21	1.73±0.20	89.97±8.64	65.79±5.23
HER-2 –	42	8.59 ±0.62	6.53±1.10	1.49±0.12	79.38±5.11	65.29±3.84

** Significant difference from patients with Grade 1 tumour (P=0.035)*
*** Significant difference from patients with tumour, which is smaller than 2 cm (P=0.007)*

Table 5 presents the correlation coefficients between serum levels of various parameters among the patient group. The analysis reveals significant relationships, particularly highlighting the positive correlations between serum TRAIL levels and both DR5 and CCL5. Specifically, TRAIL demonstrates a moderate positive correlation with DR5 (r=0.354, p=0.005) and CCL5 (r=0.347, p=0.006), suggesting that as TRAIL levels increase, there is a concomitant rise in the levels of these receptors and chemokines.

Table 5: Correlation between highlighted parameters among the patient group

	TRAIL (ng/mL)	DR4 (ng/mL)	DR5 (ng/mL)	CCL2 (pg/mL)	CCL5 (ng/mL)
TRAIL	1	-0.146	**0.354***	-0.008	**0.347****
DR4		1	-0.037	-0.220	0.025
DR5			1	-0.001	0.116
CCL2				1	0.177
CCL5					1

** Significant correlation (P=0.005)*

***Significant correlation (P=0.006)*

DISCUSSION

Breast cancer is a heterogeneous disease with different subtypes that have distinct prognoses. TRAIL and its receptors are part of the extrinsic pathway of apoptosis and play a key role in maintaining the balance between cell death and proliferation. However, the diagnostic and prognostic value of TRAIL and its receptors in breast cancer remains unclear. In this context, the current study aimed to investigate to serum levels of TRAIL, DR4, DR5, CCL2, and CCL5 in breast cancer.

The present study did not reveal any significant differences in serum TRAIL levels between breast cancer patients and the control groups, suggesting that TRAIL may not serve as a reliable biomarker for differentiating between these populations. In the existing literature, there is no consensus regarding the association of serum TRAIL levels with breast cancer pathogenesis, despite the belief that low serum TRAIL levels in cancer are typically linked to a poor prognosis and lower survival. Le Cornet et al. (9) evaluated the effect of TRAIL on breast cancer development and progression and observed no association between TRAIL and any outcome. There are no significant differences in serum TRAIL levels between patients with breast cancer and control groups in the study by Celik et al. (10), while Papila et al. (11) demonstrate significantly lower sTRAIL levels in patients with breast cancer compared to the control group.

Death receptors DR4 and DR5, part of the TRAIL pathway, are key regulators of apoptosis, and their serum levels have been widely studied for their diagnostic and prognostic value in various cancers. In the present study, elevated levels of serum DR4 and DR5 were observed in breast cancer patients compared to the control group. Serum levels of DR5 have been found to be significantly elevated in patients with ovarian, small cell lung, colorectal, and non-small cell lung cancers compared to control groups (4, 5, 12, 13). Mielczarek-Palacz et al. (5) reported that patients with ovarian cancer exhibited significantly higher levels of DR4 compared to the control group. The findings of the current study are consistent with previous findings in ovarian, small cell lung, colorectal and non-small cell lung cancer (4, 5, 12, 13). An increase in serum DR4 and DR5 levels may be associated with the enhanced release of these receptors from the cell membrane into the intracellular fluid.

In the present study, we found a positive correlation between serum sTRAIL levels and serum DR5 levels in breast cancer patients, suggesting that higher sTRAIL levels may enhance the activity of DR5 in mediating apoptosis. In contrast, no significant association was observed between sTRAIL and DR4 levels. This differential relationship implies that DR5 may play a more prominent role in apoptosis signalling pathways in breast cancer, potentially influencing tumour behaviour and response to therapy.

Chemokines secreted by tumour cells act as growth factors, inducing metastasis, angiogenesis, and immunosuppressive microenvironment formation (14). Expressions of chemokines and their receptors are effective in breast cancer metastasis (15). In our study, we found no significant difference in serum CCL2 levels between the study groups. Previous studies have reported varying results regarding serum CCL2 and CCL5 levels in breast cancer pathology. In the study conducted by Autenshlyus et al. (16), significantly lower levels of CCL2 were observed in the tumour supernatants of patients with invasive ductal carcinoma compared

to those with fibroadenoma. CCL2 serum levels were evaluated between patients with breast cancer, ductal carcinoma in situ I–III, and benign breast lesions and in healthy women that is no differences were found. However, breast cancer patients with increased CCL2 levels were correlated with advanced tumour stage and lymph node involvement (17). Plasma CCL2 levels were found to be significantly higher in breast cancer patients than in the control group (18). In our study, the cohort consisted of 62 patients with various subtypes and grades of breast cancer, predominantly invasive ductal carcinoma (IDC). This may account for the absence of differences in serum CCL2 levels, as our patient population exhibited distinct characteristics such as a wider range of tumour size compared to those reported in the literature. Furthermore, discrepancies in serum CCL2 levels may arise from variations in sample sizes and methodologies employed in different studies. These factors underscore the need for further research to elucidate the role of CCL2 in breast cancer pathology.

In our study, we found no significant difference in serum CCL5 levels between the study groups. Fujimoto et al. (19) reported significantly elevated serum levels of CCL5 in patients with metastatic breast cancer compared to those with invasive breast cancer, suggesting a potential role for CCL5 in cancer progression and metastasis. In contrast, Smeets et al. (20) found no significant changes in CCL5 levels among patients with lymph node involvement, indicating that CCL5 may not consistently reflect tumour burden across different breast cancer phenotypes. While previous studies report significant changes in serum CCL5 levels among breast cancer patients, our cohort of 62 predominantly IDC cases showed no such association. Potential explanations include differences in tumour characteristics and sample populations. For instance, Fujimoto et al. included a broader cohort, including metastatic cases, whereas our study focused solely on patients with varying grades and subtypes, predominantly IDC, which may have influenced the serum CCL5 levels. Moreover, the sample size and demographic characteristics, such as age, may differ across these studies, contributing to the divergent findings regarding CCL5. Previous research has established that TRAIL (TNF-related apoptosis-inducing ligand) enhances the production of chemokines, including CCL2 and CCL5, in breast cancer cell lines such as MCF7 (21). This aligns with our study's finding of a positive correlation between serum TRAIL and CCL5 levels in breast cancer patients, suggesting that elevated TRAIL may contribute to an increased inflammatory response within the tumour microenvironment. This correlation indicates a potential interplay between apoptotic signalling and chemokine production, which could facilitate tumour progression. Furthermore, it may impact therapeutic responses in breast cancer by creating an immune suppressive environment that hinders the effectiveness of immunotherapies and promotes resistance to treatments targeting tumour apoptosis.

A case-control study indicated an increased risk of breast cancer associated with early menarche (22). More recently, a 2024 study (23) has provided further insights, revealing that early menarche is linked to more aggressive molecular tumour characteristics among women with breast cancer. In our study, we found a significantly lower mean age at menarche in patients with grade 3 breast cancer compared to those with grade 2. This suggests that age at first menarche may influence breast cancer prognosis, underscoring the necessity for further research to understand its implications on tumour biology and patient outcomes.

Consistent with the findings of Inwald et al. (24), our study demonstrated a correlation between elevated Ki67 expression and higher tumour grade. Furthermore, we observed that low Ki67 expression was more prevalent in patients with oestrogen receptor (ER) and progesterone receptor (PR) positivity compared to those with negative hormone receptor status.

While the present study provides valuable insights into the potential diagnostic and prognostic value of serum biomarkers in breast cancer, several limitations should be considered. First, the sample size was relatively small, and larger studies are needed to confirm the findings in this study. Second, the study was limited to the evaluation of a select group of biomarkers, and ot-

her potential biomarkers may also be relevant for breast cancer diagnosis and treatment.

Third, the present study did not evaluate the potential impact of treatment on serum biomarker levels, and future studies should investigate the potential role of these biomarkers as predictors of treatment response. Moreover, the study's scope is limited by the exclusion of patients with acute infectious diseases, thereby hindering the practical implication of serum TRAIL and its receptors in the diagnosis of breast cancer.

Apoptosis plays a crucial role in breast cancer, with TRAIL and death receptors being recently studied for their potential diagnostic value. Additionally, CCL2 and CCL5 are implicated in breast cancer pathogenesis and prognosis. Serum levels of DR4 and DR5 were significantly higher in breast cancer patients than in the control group, while there was no significant difference in TRAIL levels. It is necessary to conduct additional studies with a larger sample size to establish a definite correlation between TRAIL and the diagnosis and prognosis of various subtypes of breast cancer.

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